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ORIGINAL ARTICLE

Effect of Indoxacarb on some biological and biochemical aspects of *Spodoptera littoralis* (Boisd.) larvae

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Abstract The efficacy of Avaunt (Indoxacarb 15% EC) on newly ecdysed 2nd and 4th instar *Spodoptera littoralis* (Boisd.) larvae was evaluated. Results of the conducted bioassay showed that 2nd instars were more susceptible than the 4th instars as the LC_{50} and LC_{90} values were 0.63 and 3.1 ppm for 2nd instar larvae and 2.0 and 18.75 ppm for 4th instar larvae, respectively. The total development period of the subsequent larval instars following the treatment of 2nd or 4th instars by their respective LC_{50} Indoxacarb was significantly extended by 2.8 and 3 days, respectively. Furthermore, percentage pupation and adult emergence were significantly less than their equivalent control. The biochemical study carried out on 2nd and 4th instar larvae, 24 h following their feeding on castor oil bean leaves treated by their determined LC_{50} of Indoxacarb, showed that the treatment of 2nd instar larvae caused a 32.6% and 82.7% decrease in the content of carbohydrates and lipids in larvae than their value in the control. These two respectively mentioned components were also reduced in treated 4th instar larvae by 56.4% and 76.5% than their control. Although, total protein was slightly increased by 8.7% in 2nd instars following treatment, it was found to decrease by 24.9% in treated 4th instar than that in untreated insects. The disturbance in the carbohydrate level was expressed by impairment in the activity of carbohydrate enzymes in treated 2nd and 4th instar larvae. In both treated instar larvae there was a significant increase in the enzyme activity of alpha and beta esterase as well as in glutathione *S*-transferase. Meanwhile, a significant decrease in the enzyme activities of both acetyl choline esterase and acid phosphatase was recorded in treated larvae.

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Introduction

The Egyptian cotton leafworm *Spodoptera littoralis* is a polyphagous insect of economic importance with a wide range of host plants. Chemical insecticides are an effective mean for the control and prevention of major damage caused by this insect pest. However, the extensive and continuous use of traditional insecticides creates environmental contamination and could lead to development of insect resistance. Reduced pesticides risks are considered to be safer for human health and the

environment and therefore are in constant demand as new control agents. The relatively novel oxadiazine insecticide Avaunt (Indoxacarb 15% EC), exhibits a new mode of action as it works as a sodium channel blocker resulting in paralysis and death of targeted pests. This insecticide has been reported to have a good field activity against a number of Lepidoptera as well as certain Homoptera and Coleoptera insects and exhibits reduced pesticide risk with low mammalian toxicity (Wing et al., 2000; McKinley et al., 2002).

The objective of the present work is to evaluate the efficacy of Avaunt (Indoxacarb 15% EC) as a chemical control agent against the Egyptian cotton leafworm *S. littoralis* and its effect on some biological and biochemical aspects of treated larvae.

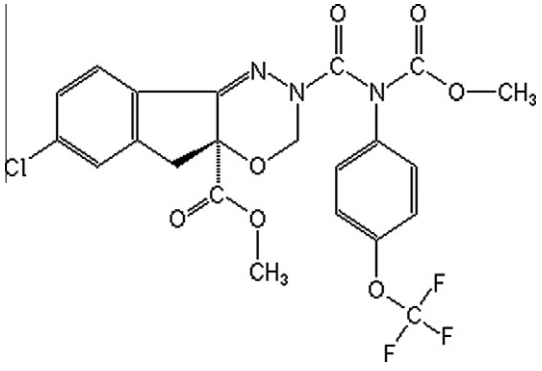
Materials and methods

Maintenance of S. littoralis culture

The original colony of the cotton leafworm *S. littoralis* was obtained from a well established culture at the Department of cotton leafworm (PPRI). The insects were maintained under laboratory conditions of 27 ± 2 °C, 70 ± 5% R.H. and 12:12 (L:D) photoperiod. Larvae were reared on fresh castor bean oil leaves, *Ricinus communis*, supplied daily in sufficient amounts, maintenance of the different developmental stages were conducted according to method described by Gamil (2004).

Tested compound

Common name: Indoxacarb 15% EC.
Trade name: Avaunt (DuPont).
Chemical name ***S*-methyl-7-chloro-2,5-dihydro-2-[|(methoxycarbonyl)[4-(trifluoromethoxy)phenyl]amino|carbonyl]indeno** [1,2-e] [1,3,4] oxadiazine-4a(3H)-carboxylate.
Chemical structure:



Bioassay

To assess the activity of Indoxacarb 15% EC a series of concentrations were prepared in distilled water which were 18.75, 9.37, 4.68, 2.30, 1.17, 0.58, 0.29 and 0.14 ppm. The dipping technique was adopted, where fresh clean castor oil leaves were immersed in one of the tested concentrations, the leaves were then allowed to dry at room temperature before being offered to newly ecdysed 2nd and 4th instar *S. littoralis* larvae. Larvae were fed on treated leaves for 24 h. Each considered concentration comprised 10 larvae and was replicated five times (i.e. 50 larvae/treatment). A similar number of larvae were considered as a control in which larvae were offered castor oil leaves immersed in distilled water.

Mortality was recorded after 24 h. Mortality percentage was corrected by Abbott formula (Abbott, 1925). Results were presented graphically as log/probit regression lines and LC_{50} and LC_{90} values and slope were calculated according to Finney (1972) and by the use of the computer program Sigma Plots for Windows (version 11).

Biological studies

Newly ecdysed 2nd and 4th instar *S. littoralis* larvae (12–24 h old) were offered castor bean oil leaves treated with Indoxacarb at their determined LC_{50} for 24 h after which time larvae were offered untreated leaves. Each larval instar comprised 10 larvae and was replicated 10 times (i.e. 100 larvae/treatment). The same numbers of larvae were considered as a control, these larvae were offered castor oil leaves immersed in distilled water. The following parameters were recorded:

(i) Larval instars duration, from the initial treated instar up to pupation.
(ii) Percentage of pupation.
(iii) Percentage adult emergence.

Biochemical bioassay

After 24 h following the feeding of 2nd and 4th instar *S. littoralis* larvae on castor bean oil treated with Indoxacarb at their determined LC_{50}, any surviving larvae exhibiting toxic symptoms were selected. The larvae of each instar were anaesthetized and rinsed with 5 ml acetone to remove surface residues, the larvae were weighed then homogenized in phosphate buffer (pH 7) using a Teflon tissue homogenizer surrounded by crushed ice. The homogenates were centrifuged at 8000 rpm for 20 min at 4 °C and the supernatant was used directly for the determination of the following:

Main contents

(i) Total carbohydrates according to Singh and Sinha (1977).
(ii) Total lipids according to Knight et al. (1972).
(iii) Total soluble protein as described by Bradford (1976).

Enzymes assay

The following enzymes activity was determined:

(i) Carbohydrates hydrolyzing enzymes; amylase, trehalase and invertase were determined by the method of Ishaaya and Swirski (1976), using starch, trehalose and sucrose as substrates.
(ii) Acetyl choline-esterase activity was determined using acetylcholine bromide (AChBr) as substrate according to the method described by Simpson et al. (1964).
(iii) Non-specific α and β esterase activity was measured as described by Van Asperen (1962) using α naphthyl acetate and β naphthyl acetate, respectively, as substrates.
(iv) Acid and alkaline phosphatase activity was measured from the larval hemolymph as described by Laufer and Schin (1971).

(v) Glutatione *S*-transferase activity (GST) was determined spectophotometrically at 340 nm according to the method of Habig et al. (1974).

Statistical analysis

Data from all experiments were subjected to the analysis of variance using the computer program SAS.

Results

Toxic effect of Indoxacarb against 2nd and 4th instar S. littoralis larvae

The conducted bioassay to determine the toxicity of Indoxacarb on 2nd and 4th instar *S. littorallis* larvae showed that 2nd instar larvae were more susceptible than the older 4th instars. As seen in Fig. 1 and Table 1, LC_{50} and LC_{90} values were 0.63 and 3.1 ppm for 2nd instar larvae and 2.0 and 18.75 ppm for 4th instar larvae, respectively. The slope values were 1.8 and 1.2 for the respective mentioned instars larvae showing more homogeneity in treated 2nd than 4th instar larvae.

Treated insects exhibited symptoms of toxicity starting by sluggish slow movement, cessation of feeding followed by regurgitation, tremor of the larval thoracic legs and mouthparts followed by insect paralysis then death. The toxic signs were dose dependent, as they were quite rapid with the higher concentrations and slower with the lower concentrations.

Effect of Indoxacarb at LC_{50} concentration on some biological aspects of S. littoralis treated as 2nd and 4th instar larvae

Biological effects of the calculated LC_{50} level of Indoxacarb on 2nd and 4th instar *S. littoralis* larvae are shown in Table 2. Following treatment with the determined LC_{50} of Indoxacarb, the total development time of the subsequent larval instars in 2nd and 4th instars was significantly extended by approximately 2.8 and 3.0 days, respectively. Of the surviving larvae

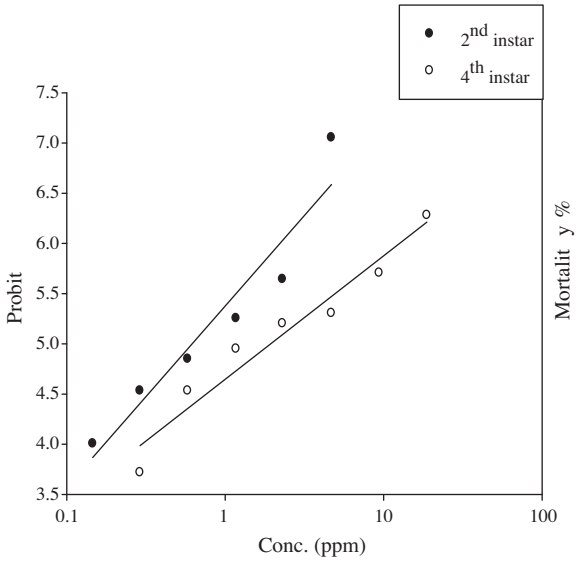


Fig. 1 Toxicity regression lines of Indoxacarb 15% EC against 2nd and 4th instar *Spodoptera littoralis* larvae.

Table 1 Toxicity values of Indoxacarb on 2nd and 4th instar *S. littoralis* larvae.

Calculated value	2nd instar	4th instar
LC_{50} (ppm)	0.63	2
LC_{90} (ppm)	3.1	18.75
Slope	1.8	1.2

50% and 42% successfully pupated in the respective mentioned instars, as compared to between 92% and 95% in untreated larvae. The duration of the pupal stage was not significantly affected when 4th instar larvae were treated; meanwhile it was significantly shortened by 1 day when 2nd instar larvae were treated. Percentage adult emergence was 78% and 83.3% of pupae obtained from treated 2nd or 4th instar larvae, respectively, these percentages were 91.3% and 93.7% in their equivalent control.

Biochemical effects

Effect on main contents

As seen in Table 3, treatment of 2nd instar *S. littoralis* larvae with LC_{50} of Indoxacarb caused a reduction in the total carbohydrates from 4.01 to 2.7 mg/ml, giving a 32.6% decrease than their value in the control. Also, a marked reduction of 82.7% in lipids content, as its value was reduced from 16.18 in the control to 2.8 mg/ml in treated 2nd instar larvae. These respectively mentioned two components were also reduced, respectively by 56.4% and 76.5% than their control, in 4th instar larvae treated by LC_{50} of Indoxacarb.

Meanwhile, total protein content was slightly elevated from 7.79 to 8.7 mg/ml (i.e. 8.7% increase) following the treatment of 2nd instars. Meanwhile, in the treatment of 4th instar larvae, total protein was significantly lowered by 24.96% (i.e. from 38.95 mg/ml in the control to 29.23 mg/ml in treated larvae).

Enzyme assay

Effect on carbohydrate enzymes activity. As seen in Table 4, treatment of 2nd instar larvae with LC_{50} of Indoxacarb caused a significant 30.8% reduction in amylase activity than that in untreated larvae. However, activities of both trehalase and invertase were significantly increased by 25.12% and 45.47% than the control, respectively. Meanwhile; treatment of 4th instar larvae caused an increase of 28.45% and 49.32% in amylase and trehalase activities, respectively, and a mild decrease of 4.3% in invertase activity than that in untreated larvae.

Effect on acetyl choline esterase, alpha and beta esterase activity. As seen in Table 4, acetyl choline esterase activity in untreated 4th instar *S. littoralis* larvae was 849.02 µg AchBr/ml/min which was significantly higher than that recorded in 2nd instar larvae, (i.e. 102.15 µg AchBr/ml/min). As a result of treatment with LC_{50} of Indoxacarb to either 2nd or 4th instars, the activity of acetyl choline esterase was reduced by nearly the same percentage, i.e. 31.97% and 30%, respectively, than that of their equivalent control.

The activity of alpha and beta esterase in *S. littoralis* 2nd and 4th instar larvae for 24 h following treatment with the calculated LC_{50} of Indoxacarb is shown in Table 4. The activity

Table 2 Duration of larvae (from initial treated instar up to pupation), percentage pupation and adult emergence of 2nd and 4th instar of *S. littoralis* (Boisd.) larvae treated with respective LC_{50} of Indoxacarb.

Biological parameters	2nd instar		4th instar	
	Treated	Control	Treated	Control
Larval duration[a] (days ± S.E)	14.58* ± 0.2	11.75 ± 0.04	13.9* ± 0.4	10.9 ± 0.06
% Pupation	50	92	42	95
Pupal duration (days ± S.E)	8.8 ± 0.1	9.89* ± 0.09	8.9 ± 0.9	8.98 ± 0.086
% Adult emergence	78	91.3	83.3	93.7

[a] From initial treated instar up to pupation.
* Mean significant difference between treated and control.

Table 3 Total carbohydrate, protein and lipid content in 2nd and 4th instar larvae of *S. littoralis* 24 h following treatment with LC_{50} concentrations of Indoxacarb.

Component (mg/ml)	2nd Instar			4th Instar		
	Control	Treated	% Increase or decrease than control	Control	Treated	% Increase or decrease than control
Carbohydrate	4.01* ± 0.010	2.7 ± 0.013	−32.6	17.33* ± 0.013	7.55 ± 0.018	−56.4
Proteins	7.79 ± 0.26	8.47* ± 0.011	+8.7	38.95* ± 0.019	29.23 ± 0.018	−24.96
Lipid	16.18* ± 0.013	2.8 ± 0.005	−82.7	28.37* ± 0.002	6.66 ± 0.010	−76.5

* Mean significant difference between treated and control.

of alpha esterase in treated 2nd instar larvae was 517.3 µg α-naphthol/ml/min/g larval weight as compared to 278 µg α-naphthol/ml/min/g larval weight in the control, being an increase by 86.1%. Beta esterase activity was also increased by 20.5% in larvae treatment as 2nd instar larvae than their control; this percentage was much lower than the increase of alpha esterase.

Similarly, treatment of 4th instar larvae with LC_{50} of Indoxacarb caused a marked increase in alpha esterase activity than that recorded in untreated larvae by 61.6%. Meanwhile, beta esterase was reduced in treated 4th instars, i.e. 44% than that recorded in untreated larvae.

Effect on acid and alkaline phosphatase. In untreated 4th instar larvae, the activity of acid phosphatase was higher than that in 2nd instar, larvae being 1.9 and 7.11 µg phenol/ml/min, respectively. Similarly the activity of alkaline phosphatase was much higher in 4th instar larvae (i.e. 95.88 µg phenol/ml/min), as compared to 13.02 µg phenol/ml/ min in 2nd instar larvae.

As seen in Table 4, acid phosphatase activity was mildly and insignificantly reduced from 1.9 to 1.6 µg phenol/ml/min in 2nd instar larvae following their treatment with LC_{50} Indoxacarb, making a 15.79% decrease. Following the treatment of 4th instar larvae, this enzyme's activity was also insignificantly reduced from 7.11 to 4.04 µg phenol/ml/min (i.e. a 43.18% reduction).

Meanwhile, alkaline phosphatase activity increased in treated 2nd instar larvae by 35.56%; however, it decreased in treated 4th instars by 12.6%.

Effect on glutathione S-transferase enzymes activity. In untreated 4th instar larvae, the activity of glutathione *S*-transferase enzyme was 102.75 µmol/min/mg which was approximately 2.8-fold its value in the younger 2nd instars, being 36.25 µmol/min/mg. Following the treatment of 2nd and 4th instar larvae with their respective LC_{50} of Indoxacarb; glutathione *S*-transferase enzyme activity was significantly increased to 49 and 125.75 µmol/min/mg, respectively. This was presented by 26% and 22.38% increase in the respective mentioned instars (Table 4).

Discussion

Indoxacarb developed by DuPont is an oxadiazine insecticide which has good field activity against a number of lepidopterous pests, as well as some insects of Homoptera and Coleoptera (Wing et al., 2000). Indoxacarb was reported to be more effective following ingestion than after topical treatment (Song et al., 2011) this was correlated with its action as a sodium channel blocker insecticide. In the present work, 2nd instar *S. littoralis* larvae were found to be more susceptible than 4th instar to Indoxacarb as evident by the calculated LC_{50} and LC_{90} values. Deepti and Srivastava (2003) reported that the LC_{50} of Indoxacarb for 4th instar *S. litura* (F.) larvae was higher in 24 h old larvae than within 48 h old larvae. There was a significant increase in the total duration of the subsequent larval instars following the treatment of 2nd or 4th instar *S. littoralis* larvae with LC_{50} concentration of Indoxacarb. A symptom of toxicity as a result of treatment with this chemical was evident with tremors of the larval thoracic legs and mouthparts and with the onset of tremors insects were unable to feed. Therefore, feeding impairment of treated larvae could lead to prolongation of the larval instars and subsequently leading to a reduction in the percentage of pupation and adult emergence. Vishal et al. (2005) reported that Indoxacarb at sublethal concentration caused feeding deterrent activity of *S. litura* (F.) larvae. Similarly, Chlorpyrifos and Spinosad insecticides were reported to cause similar effect on *S. litura* (F.) treated larvae (Singh and Sohi, 2008).

Table 4 Enzyme activities in 2nd and 4th instar *S. littoralis* larvae 24 h following treatment with LC_{50} concentrations of Indoxacarb 15% EC.

Enzyme	2nd Instar			4th Instar		
	Control	Treated	% Increase or decrease than control	Control	Treated	% Increase or decrease than control
Amylase (μg glucose/ml/min)	338.23* ± 0.003	234.01 ± 0.005	−30.8	353.94 ± 0.005	454.63* ± 0.01	+28.45
Trehalase (μg glucose/ml/min)	1015.38 ± 0.013	1271.04* ± 0.028	+25.12	2260.91 ± 0.08	3376.11* ± 0.37	+49.32
Invertase (μg glucose/ml/min)	829.74 ± 0.13	1207.01* ± 0.034	+45.47	925.7* ± 0.005	885.92 ± 0.027	−4.3
Acetyl choline-esterase (μg AchBr/ml/min)	120.15* ± 0.01	81.74 ± 0.04	−31.97	849.02* ± 0.03	594.39 ± 0.14	−30
α-Esterase (μg α-naphthol released/ml/min)	278.00 ± 0.01	517.3* ± 0.2.8	+86.1	584.9 ± 0.02	945.4* ± 0.003	+61.6
β-Esterase (μg β-naphthol released/ml/min)	652.67 ± 1.5	786.5* ± 0.023	+20.50	806.01* ± 0.02	450.9 ± 0.04	−44
Acid phosphatase (μg phenol/ml/min)	1.9* ± 0.07	1.6 ± 0.02	−15.79	7.11* ± 0.02	4.04 ± 0.03	−43.18
Alkaline phosphatase (μg phenol/ml/min)	13.02 ± 0.03	17.65* ± 0.03	+35.56	95.88* ± 0.05	83.79 ± 0.013	−12.6
Glutathione *S*T (μmol/min/mg)	36.25 ± 1.1	49* ± 0.4	+26	102.75 ± 0.6	125.75* ± 0.5	+22.38

% Increase or decrease than control = treated − control ÷ control × 100.
* Mean significant difference between treated and control.

Since many toxicants show secondary actions at high concentrations (Van Eck, 1979), the median lethal concentration (LC_{50}) was used in this study, to measure the biochemical changes in the 2nd and 4th instar larvae of *S. littoralis* following treatment with Indoxacarb. Total proteins, carbohydrates and lipids are major components necessary for an organism to develop, grow and perform its vital activities. A significant decrease in the quantities of both total carbohydrates and total lipids was detected in 2nd and 4th instars treated larvae. The disturbance in carbohydrates was expressed by impairments in the activity of carbohydrate enzymes in treated larvae. A similar observation was reported in *S. littoralis* larvae exposed to the bio-insecticide Methylamine Avermactin (Dahi et al., (2009)). Meanwhile, protein content was found to increase significantly following treatment of 2nd instar larvae, but a decrease was detected following treatment of 4th instar larvae. In the 4th instar treated larvae the decline in the level of total protein content and the increase in transaminase activities could suggest mobilization of amino acids to meet energy demands in detoxification of Indoxacarb. Exposure of 5th instar *Bombyx mori* larvae to sublethal concentrations of Fenitrothion and Ethion caused depletion in total protein content that was followed by an increase in free amino acids (Nath et al., 1997). Also, Hussain et al. (2009) reported decrease in total protein contents in *Tribolium castaneum* treated with Spinosad.

In both treated instar larvae there was a significant increase in the activity of alpha and beta esterase and a decrease in AchE. This observation could suggest that it occurred as a result of the onset of paralysis and blocking of the action potential of the nervous system caused by the toxic effect of Indoxacarb larvae. Furthermore, glutathione *S*-transferase enzyme activity was increased in treated larvae, this enzyme plays a role in detoxification mechanism in insects, therefore maybe an over production of this enzyme occurred as a result of treatment. Sarita et al. (2010) and Wang et al. (2010) reported that glutathione *S*-transferase was higher in treated larvae than their control.

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